THE UTILIZATION OF FOOD IRON BY RATS, WITH PARTICULAR REFERENCE TO SEX DIFFERENCES

BY

RAYMOND SYLVESTER GLASSCOCK

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SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ANIMAL HUSBANDRY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1941

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I. Introduction

While iron represents only a small percentage of the animal body, it plays a vital role as a constituent of hemoglobin—the respiratory pigment contained in the red blood corpuscles —and is present in all protoplasm. It forms an integral part of certain tissue pigments and is associated with chemical groupings which exist in certain enzymes, accelerating their action. With a persistent iron deficiency in the ration of an animal, the hemoglobin level will ultimately drop, resulting in a condition known as nutritional anemia.

Since iron exists in foods in various combinations, it is reasonable to suppose that, because of differences in solubility in the digestive juices and in absorption, foods of the same iron concentration might vary considerably in their value as sources of iron to the body. Some iron compounds, hematin, for example, appear to be of little or no value as a source of iron to the body. It is of considerable interest, therefore, to obtain information concerning the comparative availabilities of the iron in different foods. Many experiments have been conducted with this purpose in mind. Most of these have been biological tests, chiefly concerned with hemoglobin regeneration, but in practically all cases animals of different body size were used and because the maintenance requirement for iron varies with body size, no accurate information concerning the relative efficiency of iron utilization was obtained. Because the biological tests are long, tedious, and expensive, a chemical test, using $\alpha\ \alpha'$ bipyridyl as a reagent, has been used rather widely. This test depends upon the assumption that only ionizable iron is available to the body. While this might be true, the test does not consider the changes that may be brought about in the form of the iron by the digestive juices to which the food iron is subjected. The reliability of

this chemical test has been criticized and shown not to correlate in all cases with the biological tests.

In biological tests it has become a common practice to use a pure iron salt, such as ferric chloride, as a basis of comparison. One must assume that such a salt is entirely available, and by the chemical test this is true. Even though the absorption and utilization of ferric chloride is not completely effected by the animal, its utilization is definitely high, and probably subject to a minimum of variation. For these reasons it makes a good basis for reference.

Experiments on iron absorption and excretion have presented such variable data that one hesitates to draw conclusions. Some workers believe that inorganic iron is absorbed, but not organic, or iron present as any hematin compound, while others contend that inorganic iron must be in the ferrous state. Evidence has accumulated the past few years indicating that the intestine controls the amount of iron absorbed; that is, iron is absorbed only to the extent of body requirements. Evidence has also been submitted to indicate that anemic animals absorb and utilize ingested iron more efficiently than normal animals.

It has long been known that iron is an essential component of hemoglobin, but the process involved in hemoglobin and erythrocyte formation has remained obscure. The significance of copper and cobalt in iron metabolism has been brought to light only in recent years. It has been shown that the ingestion of iron alone will not alleviate the symptoms of nutritional anemia unless copper is present in the diet. The copper apparently does not become a component part of the final product but serves as a catalyst for the hemoglobin regeneration. Cobalt has also been found essential in the treatment of certain anemic symptoms in sheep and cattle. There has been no conclusive evidence to indicate that manganese is essential for hemoglobin regeneration.

For the past several years references to sex differences have become more numerous in nutrition articles, many of

which have been confined to the efficiency of iron utilization. A number of articles have appeared which have indicated that the females utilize iron more efficiently than the males, although there is no uniformity of ideas with respect to the differences which have been obtained. A large number of such experiments have been carried out with no control of food intake, and consequently rates and amounts of gains under such conditions are almost always in favor of the males. It seems, therefore, that it would be impossible to interpret the data obtained by such a procedure with respect to sex only.

Since there is so much disagreement in the literature concerning the comparative value of different foods as sources of iron to the body, and also concerning the relative efficiency of iron utilization by males and by females, it was hoped that an investigation could be planned in such a way as to give more reliable data on these still unanswered problems.

This investigation was conducted to determine the effect of each of three foods; namely, pork liver, round steak, and raisins, as compared with ferric chloride, on the recovery of anemic rats. Since a number of investigators had reported sex differences in iron utilization, and findings at this laboratory, H. H. Mitchell and T. S. Hamilton ('37), indicated that these differences could have been brought about by variations in food consumption, experiments were outlined with an attempt to explain the results which had been obtained. Special consideration was given to variations due to sex, when the iron intake was at a sub-maximal level and ingested as a definite percentage of the diet, and with rigid control of food consumption. In addition, experiments were carried out to show the effect of increased food consumption and body gains upon hemoglobin concentrations, iron retention per unit of weight, and per unit of nitrogen. The work was not limited to hemoglobin regeneration, but involved analytical studies of various tissues and organs for iron retention. The amount and composition of gains were also taken into consideration.

II. Experimental Procedures

Albino rats were made anemic by confining in glass cages and continued milk feeding after weaning. The milk was fortified with 0.0006 percent each of copper, cobalt, and manganese on a dry matter basis. All rats were given two drops of "Nopco" cod liver oil per day, and in addition, two drops of a solution of Harris yeast concentrate which was made by dissolving in water 20 gms. of the yeast concentrate and 20 gms. of sugar. The solution was made up to 100 cc. When the anemic rats were placed on experiment they were paired according to litter, body weight, sex, and hemoglobin level. In the experiments confined to sex differences, rats of the opposite sex were paired. The fortified milk was continued as the basal diet and vitamin supplements continued as described above.

The food to be tested was added to the milk of one member of a pair to the extent of furnishing 0.001 percent iron (dry matter basis). The other member of the pair received the same amount of iron as ferric chloride. In addition, the extra energy and nitrogen furnished by the food to be tested was equalized with glucose and glycine in the diet of the ferric chloride-fed rats. By this procedure, theoretically all factors were ruled out which could make a variation within the pairs except the source of iron. In the experiments confined to sex differences 0.001 percent iron (dry basis) as ferric chloride was added to the diet of both members of the pairs during the recovery period. In one series the females were injected with follicular hormone (Schering's Progynon-B) in an effort to bring out any possible variation related to sex. The rats were fed so that pair mates received the same amounts of food; namely, milk plus supplements. However, two experiments were conducted to determine the effect of varied food intake. Hemoglobin determinations and body weights were recorded weekly.

At the completion of an experiment each rat was prepared into the following samples for iron analysis: (1) blood,

(2) gastro-intestinal tract, (3) liver and spleen sample, (4) carcass, and a sample consisting of (5) all other organs not included above. The carcasses were analyzed for both iron and total nitrogen in experiments related to sex differences.

The variations with respect to hemoglobin regeneration and total iron retention, as well as iron retention within the various tissues and organs, as described above, were determined within the pairs. The significance of these variations was determined by the application of Student's ('08) method.

III. Summary

From a comparison of milk alone, with milk plus 0.0006 percent each of copper, cobalt, and manganese on a dry matter basis, it was found that rats receiving the additional supplements were capable of maintaining their hemoglobin levels significantly above those of rats which were receiving milk alone.

An experiment conducted to determine the relative efficiency of the iron of pork liver as compared with ferric chloride, when both supplied 0.001 percent iron, indicated that the iron of pork liver was utilized to 65 percent of that of ferric chloride for retention, and gave a value of 50 percent for hemoglobin regeneration, when the iron from the two sources was assumed to be the same for maintenance requirements.

Data obtained on hemoglobin regeneration and iron retention from ferric chloride, and from round steak, when both supplied 0.001 percent iron, showed that the iron of round steak was only 43 percent as available for retention as was the iron of ferric chloride, and 1.6 percent as valuable for hemoglobin regeneration when the maintenance requirements from the two sources were assumed to be the same.

The biological data obtained in this investigation show that when raisins are fed as a source of iron to the extent of furnishing 0.001 percent of the diet (dry basis), the utilization of this iron was not efficient enough to meet the maintenance requirements of anemic rats.

A study to determine the relative efficiency of male and female rats for iron utilization showed no significant differences to exist between the sexes with respect to retention or hemoglobin regeneration when they were consuming the same diet in equal quantities; namely, milk plus 0.001 percent iron, and 0.0006 percent each of copper, cobalt, and manganese (dry basis). While the male rats grew more as indicated by body length, there was no apparent difference in their weight gains or chemical composition. A significant difference in iron content occurred in only one of the samples analyzed, that of all other organs. This difference was brought about by the additional weight contributed by the testicles of the male rats. There was no apparent difference in their total iron content, hemoglobin regeneration, iron retention, iron per unit of weight, or per unit of nitrogen of the carcasses.

A study similar to the one just discussed to determine the relative efficiency of male and female rats for iron utilization was extended until the rats were 135 days old. In addition, the females were injected with 7 units each of (Schering's Progynon-B) at 90 days of age. Two more such injections were given ten days apart. The injection of follicular hormone had no apparent effect upon the iron utilization of the female rats when the ingestion of iron was at a submaximal level, nor was there any apparent change in the utilization of iron by the female rats when they arrived at sexual maturity.

A series of two experiments was conducted to determine the effect of food intake upon the efficiency of iron utilization and chemical composition of the animals. In one experiment the males were full-fed and the females three-fourths full-fed, and in the other experiment the females were full--fed while the males were limited to three-fourths of the food intake of the females with which they were paired. Definite differences were brought about, due to the variation in food intake, which have a bearing upon the efficiency of iron utilization regardless of sex. When the food was increased, growth as indicated by body length, and body gains, increased proportionately

over the pair mate receiving a limited diet. The increased gains resulted in more total nitrogen, while the nitrogen content in relation to body weight definitely decreased.

The rats which were full-fed regardless of sex, regenerated hemoglobin more slowly, and their total iron content per unit of body weight and per unit of nitrogen was significantly decreased.

IV. CONCLUSIONS

1. Rats on a whole milk diet become anemic more slowly when copper, cobalt, and manganese are added to the diet.

2. At a level of 0.001 percent (dry basis) the relative efficiency of utilization of the iron of pork liver is only 50 to 65 percent of that of the iron of ferric chloride when the efficiency with which the iron from the two sources is assumed to be the same for maintenance requirements.

3. At a level of 0.001 percent (dry basis) of the diet, the iron of dried round steak is less than 50 percent as available for retention as the iron of ferric chloride.

4. The iron of raisins is very poorly utilized by rats suffering from nutritional anemia.

5. There is no significant difference in the efficiency of iron utilization by male and female rats growing at the same rate for hemoglobin regeneration, or retention, when the iron is ingested at sub-maximal levels (0.001 percent of the dry matter content of the diet).

6. The injection of follicular hormone (Schering's Progynon-B), into anemic female rats, which are on an iron deficient diet, has no apparent effect upon their efficiency of iron utilization, nor is there any apparent change brought about after arriving at sexual maturity.

7. Increasing the intake of food containing less than a maximal amount of iron of one member of a pair, over the other, results in the following variations regardless of sex:

(a) Growth rate, as indicated by body length, is increased over the pair mate;

(b) Body weight is increased;

(c) Total nitrogen content is increased;

(d) Percentage of nitrogen is decreased;

(e) Hemoglobin is regenerated more slowly;

(f) The total iron content of the rat per unit of body weight, and per unit of nitrogen is significantly decreased.

These findings support the theory that the more of a deficient ration that is consumed, the quicker will the results of the deficiency appear and the more severe will be the symptoms of the deficiency. They also emphasize the desirability of controlled food-intake in this type of nutrition studies.

Conclusions based upon hemoglobin concentrations, and iron retention per unit of body weight, as a criterion for the efficiency of iron utilization in comparative studies, are of little value unless the food intake and body gains have been equal. It is possible for one animal to increase in body size, and consequently blood volume, over another animal so that the total amount of iron retained in the tissues or the total amount of hemoglobin regenerated in the blood stream is actually greater than that in the smaller animal in spite of a smaller concentration of iron per unit of weight or a lower hemoglobin concentration in the blood stream. In such a case, if both animals had received the same amount of iron, the larger animal with a smaller amount of iron per unit of weight and with the lower hemoglobin concentration may have utilized his food iron more efficiently than the smaller animal with more iron per unit of body weight and with a higher hemoglobin concentration.

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